

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
Briggs and Tatum) Group Art Unit: 1645
Serial No. 09/210,747) Examiner: V. Portner
Filed: December 15, 1998) Atty. Dkt. No. 00295.77957
)

For: CONSTRUCTION OF PASTEURELLA HAEMOLYTICA VACCINES

DECLARATION UNDER 37 C.F.R. § 132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

We, Robert E. Briggs and Fred M. Tatum, declare as follows:

1. We are named as co-inventors of the subject matter disclosed and claimed in the patent application identified above. Our curriculum vitae are attached as Exhibits 1 and 2.
2. Under our direction, experiments were carried out to demonstrate that a vaccine containing *P. haemolytica* bacteria with a leukotoxin A mutation (a deletion of amino acids 34 to 378) provides calves with protective immunity against *P. haemolytica* infection.
3. To prepare vaccine for top-dressing onto food, the bacteria were grown in whole broth culture (Columbia broth, Difco), lyophilized in skim milk (Difco), and reconstituted in sterile distilled water. To prepare vaccine for injection, a whole culture of the bacteria was diluted at least 1:10 in Earles Balanced Salt Solution. No adjuvant was added to either preparation.
4. Sixteen calves were randomly assigned to 3 groups: oral vaccinates (n=5), injected vaccinates (n=5), and unvaccinated control (n=6). Oral vaccinates received vaccine

which was simply top-dressed onto a pelleted ration. Injected vaccinates received a dose of the vaccine subcutaneously in the neck. Unvaccinated control calves received no treatment. Three weeks after the first dose, vaccinates received a second similar dose.

5. One week later, all animals were challenged with virulent *P. haemolytica* placed into the lung via the trachea. Five days after challenge, all surviving animals were humanely euthanized, and their lungs were examined.

6. Unvaccinated control animals went off-feed after challenge and suffered severe fevers and/or mortality. Orally vaccinated calves exhibited no fever and remained alert and on-feed throughout the trial. Injected vaccinates exhibited a transient fever for 1-2 days but remained alert and on-feed. Thus, the vaccine protected calves against virulent lung challenge as demonstrated by milder or complete lack of clinical signs, significantly reduced lung pathology, and significant protection from mortality. Results are shown in Tables 1 and 2 (Exhibit 3).

7. A field trial was conducted in the Fall of 1998 using 100 high-risk bull and steer calves and 120 low-risk steer calves shipped to an experimental feedlot operated by New Mexico State University in Clayton, New Mexico.

8. High-risk cattle were procured by a reputable order-buyer in southwestern Arkansas from local livestock auctions. They were collected and held for 2-5 days at the order-buyer barn prior to vaccination. On the day of vaccination, the calves were run through the chute to ear-tag and to collect specimens. Even numbered calves were cut to one pen and odd numbered calves to another. The vaccine was top-dressed onto pelleted calf ration and hay in feed bunks sufficient for all odd-numbered calves to gain access. Three days later, the calves were loaded onto separate decks of a semi-trailer and trucked to the feedlot.

9. Low-risk calves were obtained from a single ranch in central New Mexico. They

were handled similarly to the high-risk calves except they were weaned and held 1-3 days prior to vaccination, and vaccine was top-dressed onto hay only. These groups were shipped to the feedlot by separate gooseneck trailers 4 days after vaccination.

10. The calves were rested overnight at the feedlot then run through the chute for specimen collection and routine treatments including vaccination (not *P. haemolytica*), worming, dehorning, and castration. The calves were semi-randomly sorted to 18 pens which separated vaccinates from unvaccinated controls. Weekly thereafter for a total of 28 days the calves were run through the chute for collection of specimens and weight data. All feed was weighed into and out of bunks for assessment of Dry Matter Intake (DMI).

11. The performance of the calves was likely influenced by two periods of adverse weather. The high-risk calves experienced the remnants of a tropical storm immediately prior to shipment. Three weeks into the study, another storm adversely affected all the calves. Three high-risk calves, all non-vaccinates, were identified by the order-buyer as very sick at the time of loading. These calves died in transit to the feedyard. Within 3 days, 5 additional high-risk non-vaccinates died. One vaccinated calf died one week into the study and another 3 weeks into the study.

12. Each of the non-vaccinated calves died of severe pneumonic pasteurellosis with lung specimens yielding high numbers of *P. haemolytica* and *P. multocida*. No *P. haemolytica* was recovered from the lung of either of the deceased vaccinated calves, though one yielded *P. multocida* and the other yielded *Mycoplasma bovis* - both organisms for which previous *P. haemolytica* infection is a predisposing factor.

13. None of the low-risk calves died over the course of the 27 day feeding. The rate of gain of low-risk vaccinates exceeded that of non-vaccinates by 0.4 pound/day (25% greater

than non-vaccinates), however. This data is consistent with a small trial conducted by Briggs et al. in 1997 where 18 head out of 104 high-risk calves were fed the vaccine. In that preliminary trial, none of the vaccinates died compared with 14% of the non-vaccinates. Calves vaccinated only with the oral vaccine gained an extra pound/day more than did surviving non-vaccinated calves.

14. Significant increases in IgA antibody against whole *Pasteurella haemolytica* antigen was detected in nasal secretions 3 days after vaccination. Similar increases in IgG antibody occurred between day 3 and day 6 post vaccination.

15. In summary, the vaccine effectively protected calves against artificial *P. haemolytica* lung challenge as well as disease in the field. In fact, a single dose during high-stress and after significant exposure protected calves within 3 days at an order-buyer barn. Interestingly, vaccination appeared to impart a significant advantage in rate of gain in the field for vaccinated low-risk calves.

16. We declare that all statements made herein of our own knowledge are true and that we believe all statements made on information and belief are true and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Feb 12, 2001

Date

Robert Briggs

Robert E. Briggs

2 Feb 01

Date

Fred M. Tatum

Fred M. Tatum